

WHAT IS CLAIMED IS:

1. A method for producing a polypeptide with altered immunogenicity or improved
5 stability, the method comprising:
- a) selecting a region of a nucleotide sequence encoding a polypeptide of interest;
 - b) diversifying the selected region of the nucleotide sequence, thereby producing a
diversified population of nucleotide sequences;
 - c) expressing polypeptides encoded by at least a subset of the diversified population of
10 nucleotide sequences;
 - d) conjugating the expressed polypeptides obtained in step c) to at least one non-
polypeptide moiety; and
 - e) selecting at least one functional polypeptide conjugate with altered immunogenicity
or improved stability.
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2. The method of claim 1, comprising selecting the region of the nucleotide sequence by
evaluating the primary or tertiary structure of the polypeptide encoded by the nucleotide
sequence.
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3. The method of claim 2, comprising evaluating the primary or tertiary structure by
computer modeling using a program selected from Modeller, WHAT IF, NACCESS, or
Biosym/InsightII.
4. The method of claim 1, comprising diversifying the selected region by one or more of
25 DNA shuffling, random mutagenesis, focused mutagenesis, and localized mutagenesis.
5. The method of claim 4, comprising diversifying by focused mutagenesis comprising
doping or spiking with a plurality of oligonucleotides.
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6. The method of claim 1, comprising performing the diversifying step recursively.

7. The method of claim 1, further comprising altering one or more nucleotides in the selected region by site-specific mutagenesis.
8. The method of claim 1, wherein the diversified population of nucleotide sequences
5 comprises at least one nucleotide sequence comprising a reduced or increased number of codons encoding amino acid residues capable of functioning as an attachment site for a non-polypeptide moiety selected from among a sugar moiety, a lipophilic molecule, a polymer molecule, or an organic derivatizing agent.
- 10 9. The method of claim 1, comprising expressing the variant polypeptides encoded by the diversified population of nucleotide sequences in a cell, which cell comprises a bacterial cell, a fungal cell, a plant cell, an animal cell, a mammalian cell, or a human cell.
- 15 10. The method of claim 1, comprising conjugating a non-polypeptide moiety selected from a sugar moiety, a lipophilic molecule, a polymer molecule, or an organic derivatizing agent.
11. The method of claim 1, comprising selecting the at least one functional polypeptide conjugate by a high throughput screening assay.
- 20 12. The method of claim 11, wherein the high throughput screening assay is performed in one or more microtiter plates.
13. The method of claim 1, wherein stability comprises increased functional *in vivo* $\frac{1}{2}$ life.
- 25 14. A method for producing a polypeptide with a desired property, the method comprising;
a) expressing a diversified population of nucleotide sequences encoding a polypeptide of interest;
b) glycosylating at least one polypeptide expressed in step (a) *in vivo* or *in vitro*;
30 c) selecting at least one polypeptide with a desired property.

15. The method of claim 14, wherein the diversified population of nucleotide sequences is produced by one or more of DNA shuffling, random mutagenesis, focused mutagenesis, localized mutagenesis, and site specific mutagenesis.
- 5 16. The method of claim 14, comprising diversifying by focused mutagenesis comprising doping or spiking with a plurality of oligonucleotides.
17. The method of claim 14, wherein the diversified population of nucleotide sequences comprises at least one nucleotide sequence comprising a reduced or increased number of
10 codons encoding amino acid residues capable of functioning as a glycosylation site *in vivo* or *in vitro*.
18. The method of claim 14, comprising identifying the at least one nucleotide sequence by a high throughput screening assay.
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19. The method of claim 15, wherein the high throughput screening assay is performed in one or more microtiter plates.
20. The method of claim 14, wherein the desired property is altered immunogenicity or
20 improved stability.
21. The method of claim 20, wherein improved stability comprises increased functional *in vivo* $\frac{1}{2}$ life.
- 25 22. The method of claim 14, wherein the selected polypeptide has an altered glycosylation pattern relative to the polypeptide of interest.
23. A method for altering immunogenicity or improving stability of a polypeptide of interest, the method comprising:
30 a) expressing a diversified population of nucleotide sequences encoding the polypeptide of interest;

b) blocking at least one functional site of a variant polypeptide expressed in step a) with a helper molecule;

c) conjugating one or more non-polypeptide moieties to the blocked polypeptide of step b); and

5 d) identifying at least one variant polypeptide with altered immunogenicity or improved stability.

24. The method of claim 23, wherein improved stability comprises increased functional *in vivo* $\frac{1}{2}$ life.

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